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(54) Title of the Invention: **Fluorescent Immunoassay Container**

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SPECIFICATION

1. Title of the Invention

Fluorescent Immunoassay Container

2. Claims

(1) A fluorescent immunoassay container, said container holding a sample that emits fluorescent light, wherein a layer that reflects fluorescent light is formed on an inner layer or outer surface that does not come into contact with the sample.

(2) The fluorescent immunoassay container according to Claim 1, wherein the layer that reflects fluorescent light is composed of a metal or a substance containing a metal.

(3) The fluorescent immunoassay container according to Claim 1, wherein the surfaces that come into contact with the sample are composed of plastic.

3. Detailed Description of the Invention

Field of Industrial Utilization

The present invention relates to a sample container used in fluorescent immunoassay, which is a means for immunological testing.

Prior Art

Fluorescent analysis is widely utilized as a means for analyzing trace amounts, and fluorescent immunoassay, which is an immunological test method to which this measurement technology is applied, is today being incorporated into clinical testing. This

measurement method is a high-sensitivity immunoassay method that represents the development of a method generally called enzyme immunoassay, and is said to allow measurement of even smaller amounts than radiation immunoassay, which was considered to be the most sensitive method. In this measurement method, an antigen (or antibody) is adsorbed and fixed to the inner surface of a container, and then an antibody (or antigen) is added, an antigen-antibody reaction is progressively conducted, and finally an enzyme-labeled antibody (or antigen) is reacted. Further, a fluorescent substrate (a substance that emits fluorescent light in an enzyme reaction) is added to this enzyme fixed to the antibody, this is irradiated with excitation light, and the amount of fluorescent light emitted by the sample is read.

Japanese Laid-Open Patent Application S59-132335 discloses a container used in fluorescent immunoassay. The goal here is to detect more precisely the fluorescent light emitted by a sample by suppressing the self-fluorescence exhibited by the material constituting the container itself. More specifically, either a pigment is kneaded into the plastic material that makes up the container, or the material is coated with a pigment, the goal of which is to prevent excitation light from infiltrating the inside of a molded article, and reduce the occurrence of self-fluorescence. However, as disclosed in Japanese Laid-Open Patent Application S60-6868, when an additive such as a pigment is added to a plastic material, a problem is that this results in uneven adsorptivity of antigens (or antibodies) at the surface thereof, so this approach cannot necessarily be considered suitable for a container to be used in fluorescent immunoassay.

Meanwhile, there are many low-fluorescence materials on the market that have little self-fluorescence with almost no effect on fluorescent measurement, and measurement devices have also been improved to the point that they can handle both excitation wavelengths and fluorescence wavelengths at high precision, and any background self-fluorescence is automatically corrected. Therefore, the use of a container with low self-fluorescence is not absolutely necessary for fluorescent immunoassay, but if the fluorescent light emitted by a sample could be collected efficiently and in greater quantity, measurement at even higher levels of sensitivity would be possible even with very weak fluorescent light, and there is considerable latent need for such technology.

The inventors investigated various methods in an attempt to meet this need, whereupon they discovered that measurement sensitivity can be increased by providing a layer that reflects fluorescent light to the inside of a container. Follow-up research led to the present invention.

Object of the Invention

Specifically, the object of the present invention is to provide a fluorescent immunoassay container with which high-sensitivity measurement is possible.

Constitution of the Invention

The present invention is a fluorescent immunoassay container, said container holding a sample that emits fluorescent light, wherein a layer that reflects fluorescent light is formed on an inner layer or outer surface that does not come into contact with the sample.

The container can be in the form of a microplate, cuvette, test tube, conical centrifuge tube, reaction plate, or the like, and is selected according to the intended application, with no particular restrictions imposed thereon.

As discussed above, in fluorescent immunoassay an antigen, antibody, or other such protein is adsorbed and fixed to a container surface, but the adsorption of proteins to metal is generally unstable, and consistent reproducibility cannot be expected. In contrast, adsorption to plastics is stable, and high precision measurement can be carried out using a container with a plastic surface. Therefore, the surface to which the proteins are adsorbed must be separate from the reflective layer. A container that meets this condition can be, for example, a container as shown in Fig. 1, in which a reflective layer 2 is provided to the outer surface of a plastic molded article 1, or a container as shown in Fig. 2, in which a reflective layer 2 is provided to the inner surface of a molded container 4, and a plastic surface layer 3 is provided over this (that is, the reflective layer 2 is an inner layer).

The layer 2 that reflects fluorescent light is composed of a metal or a substance that contains a metal.

Metals that can be used in the present invention include silver, mercury, gold, platinum, copper, aluminum, nickel, tin, titanium, chromium, indium, or the like, or a mercury amalgam or an alloy of the above metals. Examples of substances that contain a metal include metal paints and silver paste in which metal microparticles are dispersed in a resin. There are no particular restrictions on how the reflective layer 2 composed of a metal or a substance containing a metal is formed on the surface of the molded container 4 or the plastic molded article 1, but examples include metal plating, vapor deposition, sputtering, ion plating, and coating with a substance that contains metals.

The method for forming the plastic surface layer 3 on the surface of the reflective layer 2 can involve coating with a plastic solution or emulsion, or dipping the container in one of these, or covering with a plastic layer by plasma polymerization or the like.

As long as it is transparent and has low self-fluorescence, any plastic can be used to form the plastic molded article 1 and the plastic surface layer 3, but favorable examples include polystyrene, polyvinyl chloride, and polymethyl methacrylate. Furthermore, there is no problem whatsoever with subjecting the surface of this plastic to a treatment that improves its adsorption of proteins such as antigens and antibodies. Also, with the present invention, since the fluorescent light is reflected and amplified through the plastic layer of the container, a thinner layer is preferable in terms of reducing fluorescent light loss within this layer.

There are no particular restrictions on the thickness of the metal layer that serves as the reflective layer 2 as long as the layer retains its reflection function. In addition to plastic, the material that forms the molded container 4 can also be glass, ceramic, metal, or the like, with no particular restrictions imposed thereon. Even when a plastic is used, there are none of the above-mentioned restrictions on self-fluorescence or transparency, and various kinds of plastic such as polyethylene and polypropylene can be used. When a metal is used, an advantage is that if its inner surface is polished to a mirror finish, there will be no need to form the reflective layer 2. Also, when the reflective layer 2 in Fig. 2 is formed using a silver paste, metal paint, or the like, it is possible for the surface thereof

to be substantially a resin layer (plastic), and to omit the formation of the plastic surface layer 3.

Effect of the Invention

High-sensitivity measurement is possible when the fluorescent immunoassay container of the present invention is used. Specifically, when the fluorescent light emitted by a measurement sample is reflected by the reflective layer in the present invention, there is an increase in the fluorescence intensity detected by the measurement device. As a result, even minute amounts that could not be accurately measured in the past because of low intensity can be measured at high precision.

The present invention will now be described in more specific terms through comparative examples and working examples.

Reference Example 1

A comparison of self-fluorescence was conducted using the polystyrene and polyvinyl chloride microplates from various companies as fluorescent immunoassay containers.

The fluorescent light measurement device was a Micro Fluor (trade name) made by Dynatech. Measurement was conducted at an excitation wavelength of 365 nm and a fluorescence wavelength of 450 nm. These wavelengths correspond to the measurement wavelengths of 4-methylunbelliferone, a typical fluorescent reagent. The results are given in Table I, which shows that the majority [of these materials] have low self-fluorescence.

Table 1

Polystyrene microplates

Type	Company S, S type	Company S, amino type	Company A	Company B	Company C
Fluorescence intensity	11	6	14	8	5

Polyvinyl chloride microplates

Type	Company D	Company E	Company F	Company G, type A	Company G, type HA
Fluorescence intensity	80	60	240	1	13

Working Example 1

A fluorescent light reflective layer was formed on a plastic container (ELISA plate, aminated type, model number MS-3696F, made by Sumitomo Bakelite, made of polystyrene). A reflective layer was formed on the outer surface of the molded article by treating a thin film of aluminum for 120 minutes at 5 mA using a sputtering apparatus made by Nihon Shinku Kogyo.

100 μL of an aqueous solution of 4-methylunbelliferone was added to each well of the plate in a concentration of 10^{-5} to 10^{-3} M. The fluorescence intensity was measured with the same fluorescent light measurement device and at the same wavelengths as in Reference Example 1. As a comparative example, the same measurements were conducted using a plate on which no reflective layer had been formed. As shown in Table 2, the fluorescence intensity was higher when the container of the present invention was used.

Table 2

Plate		Concentration (M)			
		10^{-8}	10^{-7}	10^{-6}	10^{-5}
Comp. Ex.	untreated plate	5	45	520	1039
Working Ex.	plate having reflective layer	18	238	1014	4202

Working Example 2

The outer surface of a molded article was silver plated, using a microplate made by Flow (made of polyvinyl chloride, HA type) [as the molded article].

100 μL of β-D-galactosidase (made by Sigma; hereinafter referred to as β-Gal) was added to each well of the plate in a concentration of 10^{-2} to 10^{-5} mg/mL, and the plate was allowed to stand for 18 hours at 4°C. After washing with physiological saline, 50 μL of 4-methylunbelliferyl-β-D-galactoside (made by Koch-Light) with a concentration of 3×10^{-4} M was added to each well and allowed to react for 30 minutes at room temperature. 200 μL of glycine-sodium hydroxide buffer (10.3 pH) was added to each

well to stop the reaction, and measurement was conducted in the same manner as in Working Example 1. As a comparative example, the same measurements were conducted using a plate that had not been plated. As shown in Table 3, the reflective layer of the present invention is very effective.

Table 3

Plate		β-Gal concentration (mg/mL)			
		10^{-5}	10^{-4}	10^{-3}	10^{-2}
Working Ex.	silver-plated plate	75	199	568	856
Comp. Ex.	untreated plate	32	62	154	271

4. Brief Description of the Drawings

Figs. 1 and 2 are diagrams illustrating the layer constitution in examples of the present invention. Fig. 1 is a container in which a reflective layer is provided to the outer surface of a plastic molded article, while Fig. 2 is a container in which a reflective layer is provided between (as an inner layer) a molded container and the plastic surface on the inside thereof.

Applicant: Sumitomo Bakelite Co., Ltd.

Fig. 1

2 reflective layer

1 plastic molded article

Fig. 2

2 reflective layer

3 plastic surface layer

4 molded container